

Economic Analysis of Recombinant Human Lysozyme and Hen Egg-white Lysozyme Purification

March 8, 2008

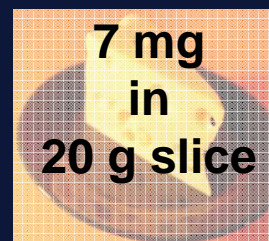
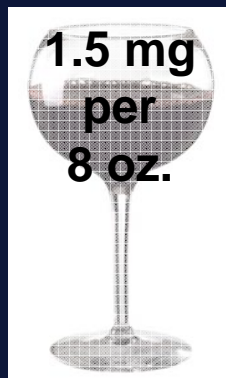
Lisa Wilken and Zivko Nikolov
Biological & Agricultural Engineering
Texas A&M University

Applications

- Recombinant Human Lysozyme from Rice
 - Cell lysis: in culture protein extraction
 - Baby formula additive, oral rehydration solution
- Hen Egg-white Lysozyme
 - Food Processing
 - Lysozyme accepted by FAO/WHO in 1993 under GMP
 - Have you consumed lysozyme lately?
 - Common preservative for wine and cheese industry

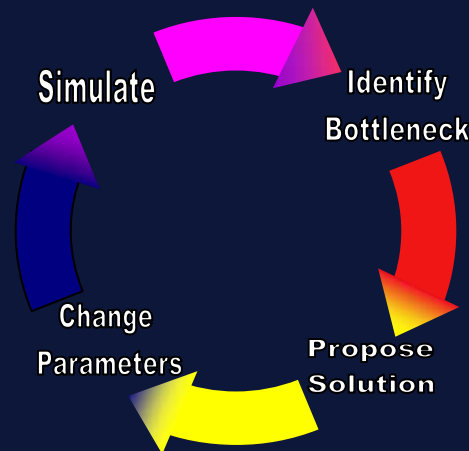


www.invitria.com

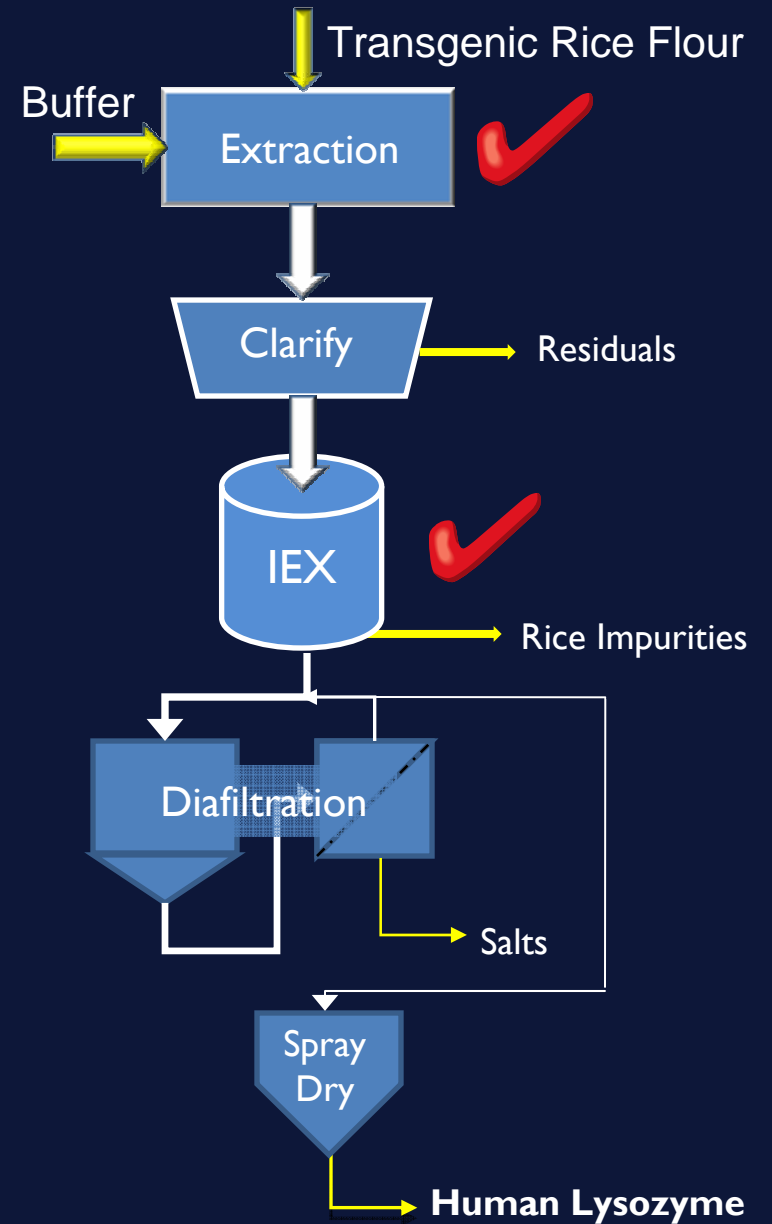
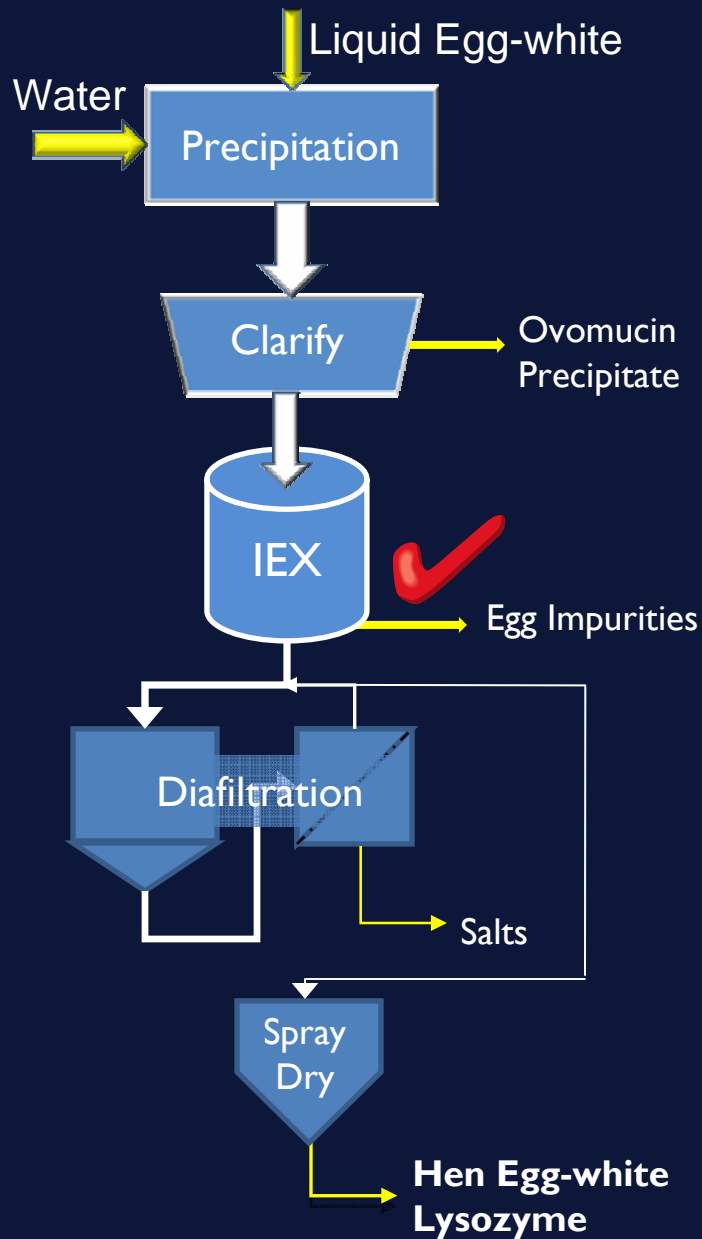


Purpose

- Determine whether human lysozyme could be economically competitive to hen egg-white lysozyme
 - 1 ton of purified lysozyme/yr
 - >90% purity
- SuperPro Designer[®] (Intelligen Inc.)
 - Tool for process development and to guide research
 - Incorporate lab data and processing knowledge into simulation
 - Simulate effect of variations in material costs and process parameters



Process Diagrams

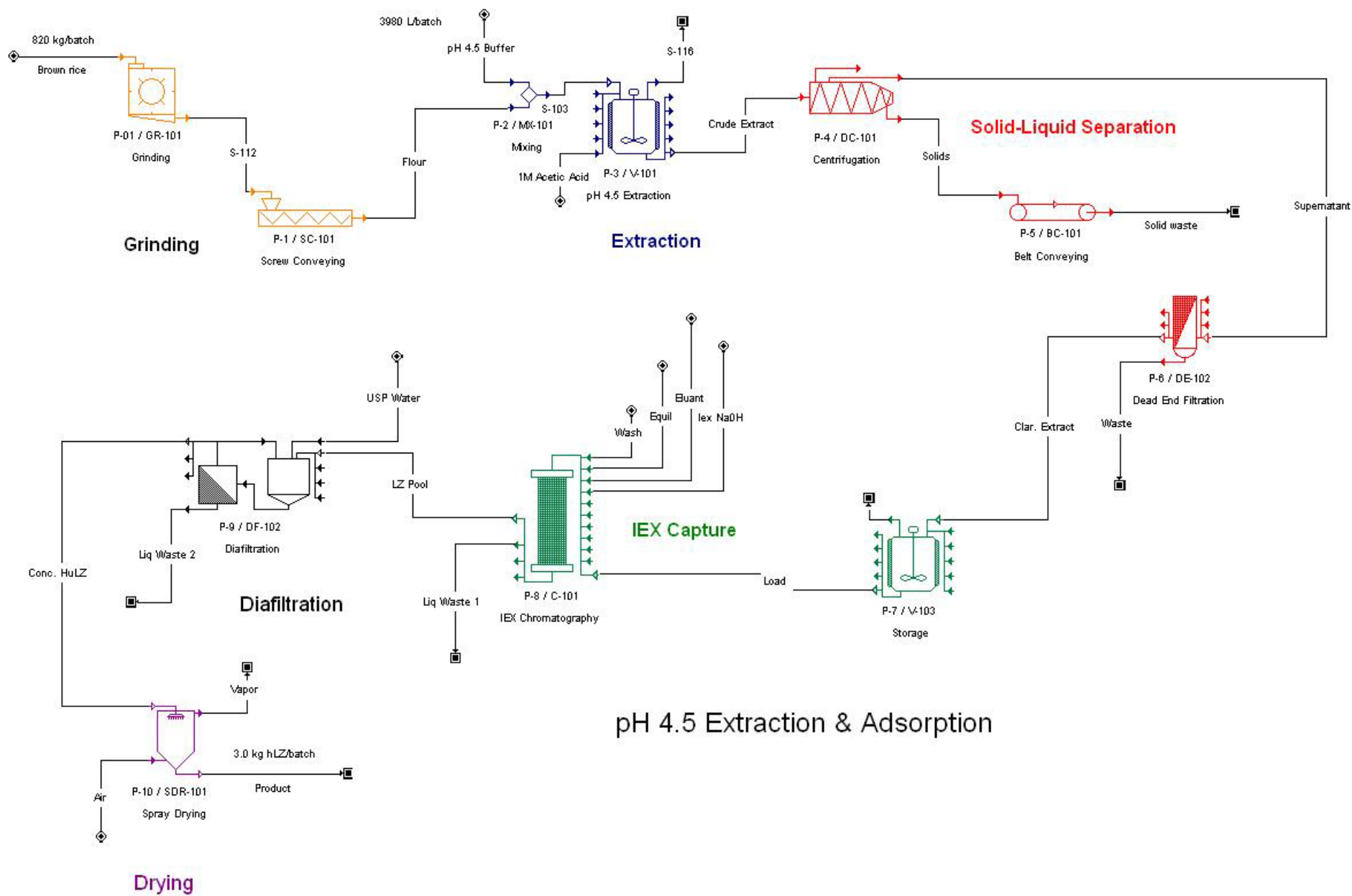


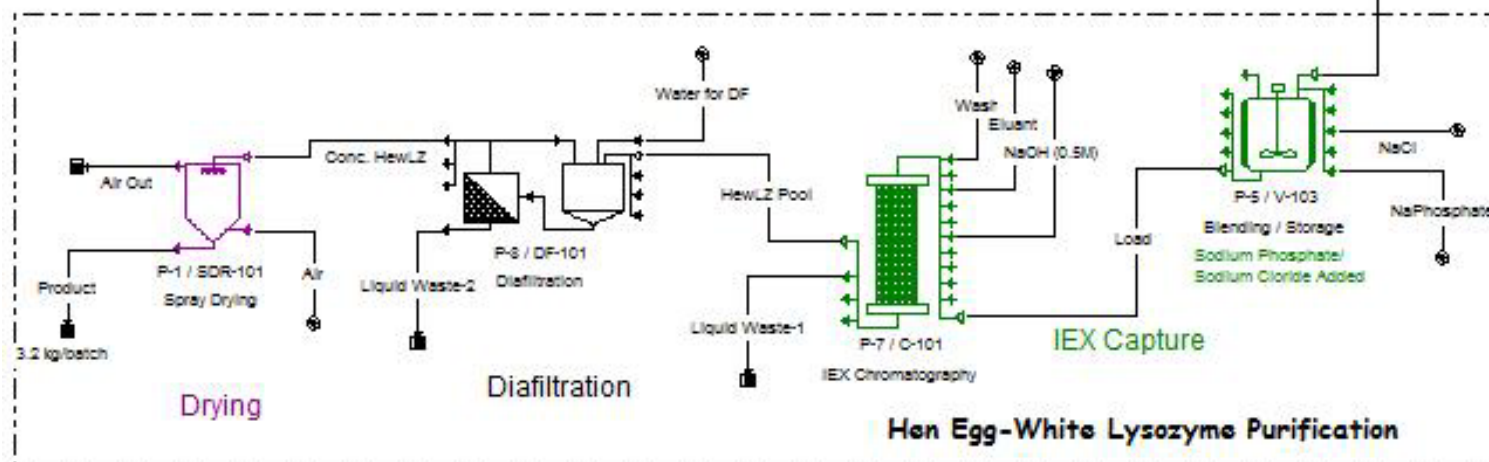
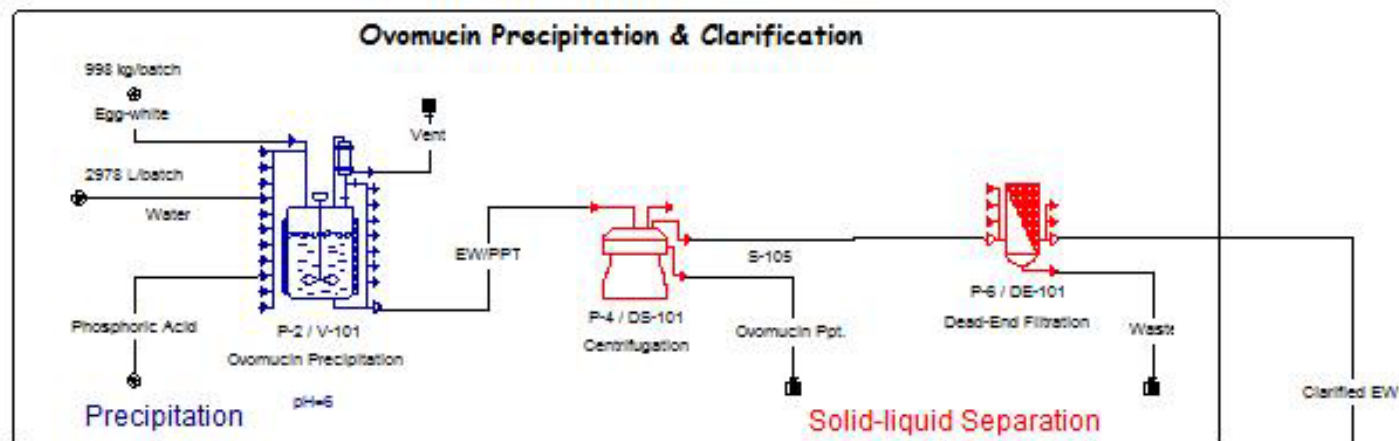


Processing Parameters

- Human Lysozyme Extraction¹
 - Maximize lysozyme recovery
 - Maximize lysozyme to native protein ratio
- Cation Exchange Chromatography
 - Binding capacity
 - Minimize column size and cost
 - Purity
 - Minimize # of purification steps
 - Yield
 - Reduce raw material requirements
 - Reduce number of batches and processing time

¹Wilken and Nikolov. 2006, 22(3) 745-752.





Cost Estimation: Raw Materials

- \$0.33/kg liquid egg white
- \$1/kg brown rice
 - Calculated from production cost
 - \$750/acre transgenic rice (assumed 2x cost of nt rice)
 - \$375/acre non-transgenic rice (USDA)
 - Brown rice 80% of rough rice
 - Cost calculated with range of yields ($\pm 20\%$)

\$/kg brown rice		
<i>low yield</i>	<i>average</i>	<i>high yield</i>
0.43	0.34	0.29

- Why cost difference?
 - Identity preservation
 - Crop isolation
 - Grow rice in Kansas?

Raw Materials

- How many acres of rice are needed for 1 ton human lysozyme/year?
 - 8-12 kg lysozyme/acre if 2200-3300 kg rice/acre yield¹
 - 85-130 acres
- How many eggs does it take to produce 1 ton of hen egg-white lysozyme/yr? How many chickens?
 - 10 million eggs or 38,400 chickens
 - 0.5 acre required²



¹ USDA Rice Yearbook 89001, ERS

² United Egg Producers Certified Welfare Guidelines

Cost Estimation

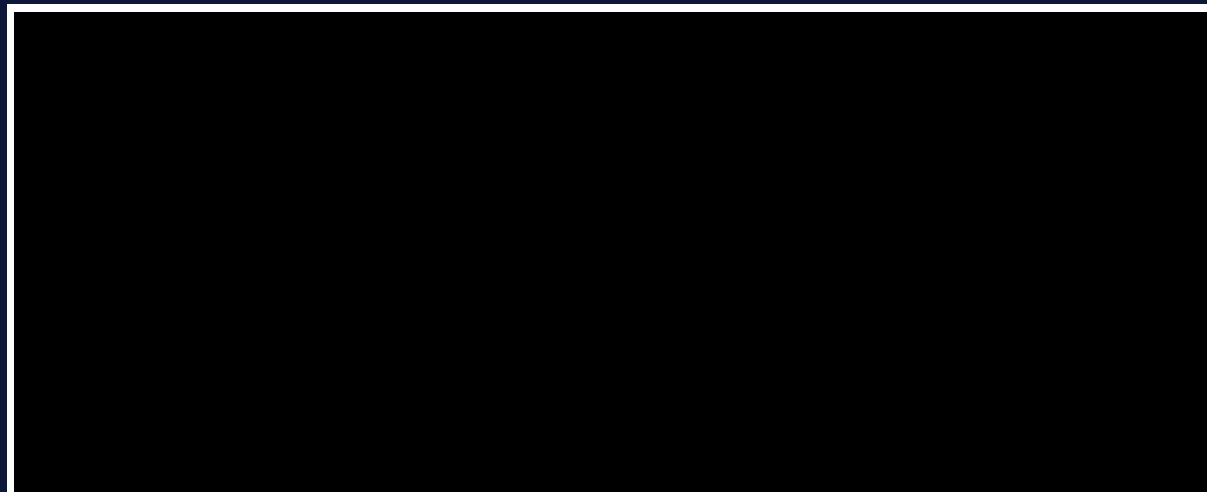
- Equipment
 - User-defined model based on quotes
 - Built-in model
- Capital investment
 - Based on equipment cost calculated using SuperPro
 - Lang factor of 3

	\$1000/yr	
	Human LZ	HewLZ
Equipment (FOB) Cost	1435	1233
Direct Fixed Capital (DFC)	4305	3699
Working capital (3% DFC)	129	111
Start-up cost (5% DFC)	215	185
Total Capital Investment (DFC+WC+Startup)	4649	3995

- Reagents
 - ICIS chemical cost

Cost Comparison

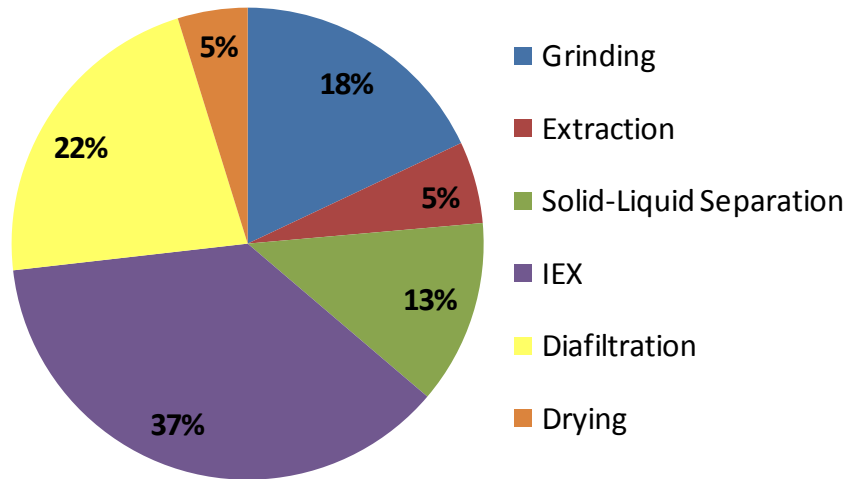
- Direct vs. Indirect Manufacturing Costs



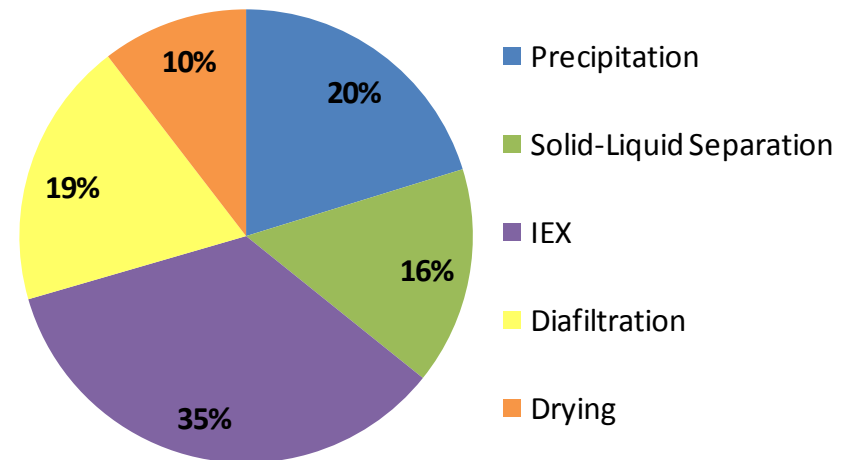
	\$/g	
	Human LZ	HewLZ
Indirect Manufacturing Cost		
Maintenance (3% DFC for this project)	0.13	0.11
Insurance (1%) & property (2%) taxes (3% DFC)	0.13	0.11
Administration (20% of operating labor)	0.14	0.14
Depreciation (10yrs)	0.42	0.36
Total Indirect Manufacturing Cost (\$/g)	0.8	0.7

Section Cost: % of Total Production Cost

Human Lysozyme



- IEX consistently most expensive!
- Evaluate major cost contributors to this section

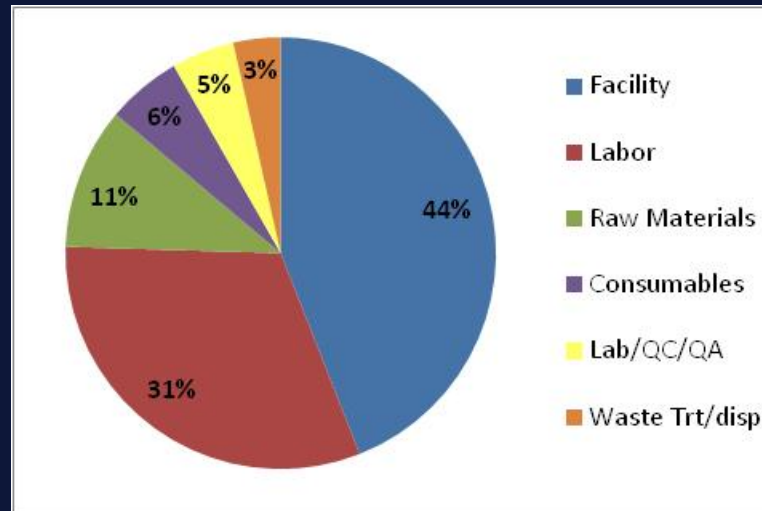


Hen Egg-white Lysozyme

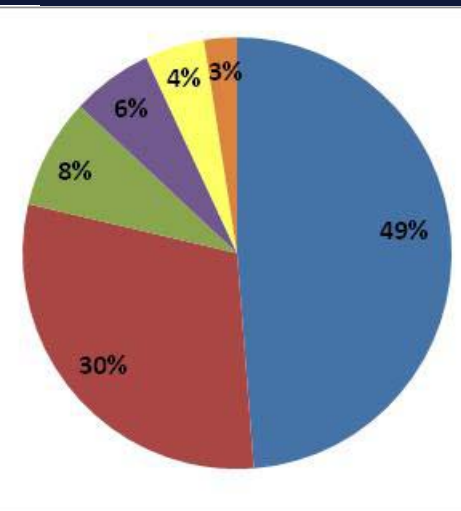
Cost Contributors

- IEX Cost Distribution

Hen Egg-white Lysozyme



Human Lysozyme

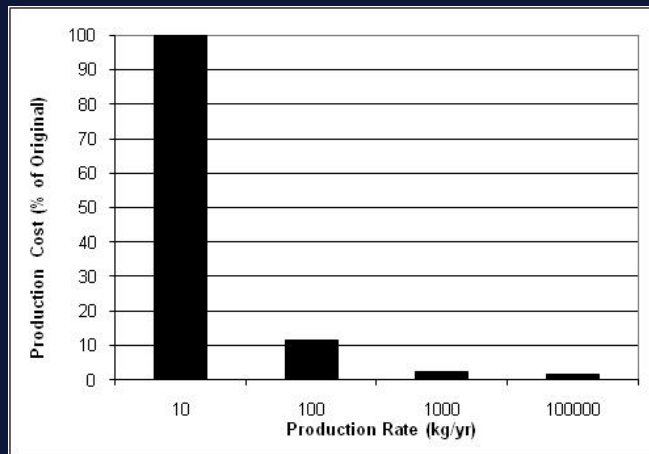


- Major Cost Contributors

- Facility-related
- Labor

Considerations & Process Improvements

- Economy of scale
 - Most important in preliminary sensitivity analysis



- Higher purity lysozyme for other applications
 - Simulated an alternative human lysozyme process to give higher final product purity
 - 8% increase in annual operating cost but potentially higher value product



Considerations

- Value of byproducts
 - Rice flour residuals for biofuels
 - Egg white protein
- Cost per unit similar for both lysozymes
 - Human lysozyme activity 3x activity of hen egg-white lysozyme
 - Application will dictate product specs, throughput, and cost



Acknowledgements

- Ventria Biosciences, Dr. Ning Huang
- Dr. Zivko Nikolov